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# Evaluation of colour pre-processing on patch-based classification of H&E-stained images

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**Abstract.** This paper compares the effects of colour pre-processing on the classification performance of H&E-stained images. Variations in the tissue preparation procedures, acquisition systems, stain conditions and reagents are all source of artifacts that can affect negatively computer-based classification. Pre-processing methods such as colour constancy, transfer and deconvolution have been proposed to compensate the artifacts. In this paper we compare quantitatively the combined effect of six colour pre-processing procedures and 12 colour texture descriptors on patch-based classification of H&E-stained images. We found that colour pre-processing had negative effects on accuracy in most cases – particularly when used with colour descriptors. However, some pre-processing procedures proved beneficial when employed in conjunction with classic texture descriptors such as co-occurrence matrices, Gabor filters and Local Binary Patterns.

**Keywords:** Colour · Histology · Hematoxylin · Eosin · Texture.

## 1 Introduction

Digital Pathology has grown considerably in recent years encompassing computer-based activities that allow for improvements and innovations in the workflow of pathology [1]. In this domain the automated processing of tissue samples has received increasing attention due to the potential applications in diagnosis [2], grading [3], identification of tissue substructures [4], prognostication and mutation prediction [5]. A number of problems, however, still limit the adoption of digital pathology on a large scale: the relatively scarce availability of large labelled datasets of histological images, the differences in the acquisition systems and/or protocol used as well as the variability in tissue preparation and/or stain reactivity [6]. The latter, in particular, can generate colour variations and artifacts that can reduce significantly the accuracy of computer-based methods. This

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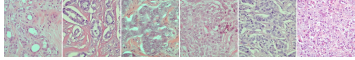
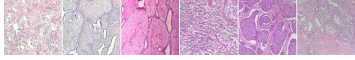
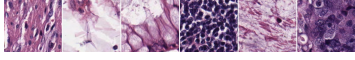
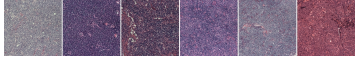

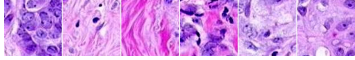
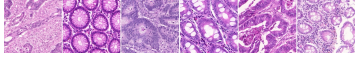
Abbrev.	Sample images	Tissue type	Classes / Patch size
AP		Breast cancer (invasive ductal carcinoma)	Grade I ( $n = 107$ ), II ( $n = 102$ ) and III ( $n = 91$ ) / $1280\text{px} \times 960\text{px}$
BH		Breast cancer (eight histological types)	Benign ( $n = 625$ ) and malignant ( $n = 1370$ ) / $700\text{px} \times 460\text{px}$
KM		Low and high grade colorectal cancer	Epithelium, stroma, complex stroma, debris, adipose, necrosis and background ( $n = 625$ each) / $150\text{px} \times 150\text{px}$
LM		Lymphoma	Chronic lymphocytic leukemia ( $n = 113$ ), follicular lymph. ( $n = 139$ ) and mantle cell lymph. ( $n = 122$ ) / $1388\text{px} \times 1040\text{px}$
NKI		Breast cancer (grades I, II and III)	Epithelium ( $n = 1106$ ) and stroma ( $n = 189$ ) / $100\text{px} \times 100\text{px}$
VGH		Breast cancer (grades I, II and III)	Epithelium ( $n = 226$ ) and stroma ( $n = 47$ ) / $100\text{px} \times 100\text{px}$
WR		Colorectal cancer	Benign ( $n = 74$ ) and malignant ( $n = 91$ ) / Variable

Fig. 1: Datasets used in the experiments: round-up table and sample images.

problem has attracted much attention lately and different colour pre-processing methods have been proposed as possible solutions [7]. Still, their beneficial effects on patch-based classification of H&E-stained images are not clear, since only few studies have addressed the subject in a quantitative way [8,9,10]. Also, apart from [10], little has been investigated as concerns the coupled effects of colour pre-processing and the specific image descriptor used.

In this paper we present a quantitative evaluation of the effects of colour pre-processing on patch-based classification of H&E-stained images. The study is based on seven datasets of histological images from different sources, six colour pre-processing procedures and 12 colour texture descriptors.

## 2 Materials and methods

### 2.1 Image datasets (Fig. 1)

**Agios Pavlos (AP).** Breast carcinoma histological images from the Department of Pathology, ‘Agios Pavlos’, General Hospital of Thessaloniki, Greece [11] (<https://zenodo.org/record/834910>) representing tissue samples from 21 patients with invasive ductal carcinoma of grade I, II and III.

**BreakHis (BH).** Breast carcinoma histological images from Pathological Anatomy and Cytopathology, Paraná, Brazil [12] of breast tumour from eight different histological subtypes (<https://omictools.com/breakhis-tool>).

**Kather Multiclass (KM).** Histological images of colorectal cancer from the University Medical Center Mannheim, Heidelberg University, Germany [4] (<https://zenodo.org/record/53169>) representing eight tissue subtypes.

**Lymphoma (LM).** Multi-center collection of histological images from malignant lymphoma [13] (<https://ome.grc.nia.nih.gov/iicbu2008/lymphoma/index.html>) organised in three classes: chronic lymphocytic leukemia, follicular lymphoma and mantle cell lymphoma.

**Netherlands Cancer Institute (NKI).** Tissue micro-arrays (TMAs) from a cohort of patients with breast cancer enrolled at the Netherlands Cancer Institute, Amsterdam, Netherlands [14] ([https://tma.im/tma\\_portal/C-Path/supp.html](https://tma.im/tma_portal/C-Path/supp.html)). The dataset includes with segmentation masks from which we extracted tiles of well defined areas of epithelium and stroma.

**Vancouver General Hospital (VGH).** Same structure as (NKI), but here the images come from a cohort of 328 patients enrolled at Vancouver General Hospital in Canada [14] ([https://tma.im/tma\\_portal/C-Path/supp.html](https://tma.im/tma_portal/C-Path/supp.html)).

**Warwick-QU (WR).** Histological images of colorectal cancer from the University Hospitals Coventry and Warwickshire, United Kingdom [15] (<https://warwick.ac.uk/fac/sci/dcs/research/tia/glascontest/download/>) organised in two classes (benign and malignant).

## 2.2 Colour normalisation

We evaluated three colour constancy, two colour transfer and one colour deconvolution method as detailed below. The effects of each method on a set of sample images are shown in Figs. 2–3.

*Colour constancy.* We considered chromaticity representation (‘chroma’ in the remainder), grey-world normalisation (‘gw’) and histogram equalisation (‘heq’) [16]. In the experiments we used Jost van de Weijer’s Color Constancy Toolbox (<http://lear.inrialpes.fr/people/vandeweijer/research.html>) and Matlab’s `histeq()` function respectively for ‘gw’ and ‘heq’.

*Colour transfer.* We employed Macenko’s [17] and Reinhard’s [18] methods using the implementation provided with Warwick’s Stain Normalization Toolbox (<https://warwick.ac.uk/fac/sci/dcs/research/tia/software/sntoolbox/>, SNT henceforth). For each of the two approaches we used four target images, denoted in the remainder as T1, T2, T3 and T4 (Fig. 2). These are all histology images except T1, which is a colour calibration checker. As for the others, T2 is part of SNT demo, whereas T3 and T4 come from The Cancer Genome Atlas (<https://cancergenome.nih.gov/>). The target images were chosen by the authors based on their subjective judgement.

*Colour deconvolution.* Colour deconvolution was based on Ruifrok and Johnston’s method [19], again via SNT. In the remainder this is denoted as ‘decoRJ’.

## 2.3 Image descriptors

*Colour histogram (FullHist).* Joint three-dimensional colour histogram [20] with ten bins per channel ( $10^3 = 1000$  features).

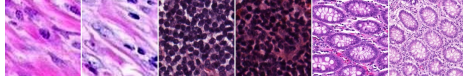
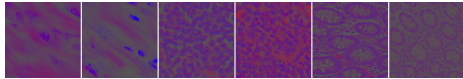
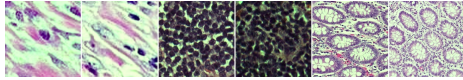
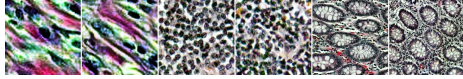
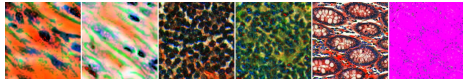

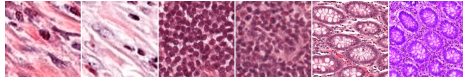

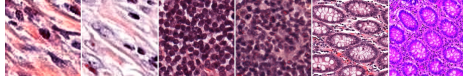

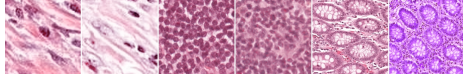

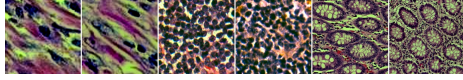

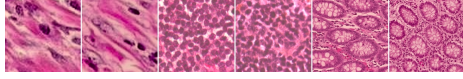

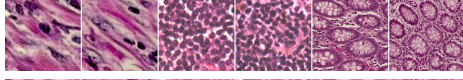

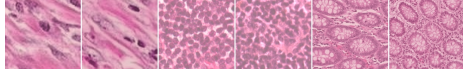

Original images					
					
Colour-normalised images			Method	Target image	
			chroma	—	
			gw	—	
			heq	—	
			Macenko		
			Macenko		
			Macenko		
			Macenko		
			Reinhard		
			Reinhard		
			Reinhard		
			Reinhard		

Fig. 2: Illustration of the effects of normalisation. Notice the influence of target images.

*Marginal colour histograms* (MargHists). Concatenation of the three 1D intensity histograms [21] of each colour channel ( $256 \times 3 = 768$  features).

*Grey-level co-occurrence matrices* (GLCM). Contrast, correlation, energy, entropy and homogeneity from GLCM computed at distance 1px, 2px and 3px and

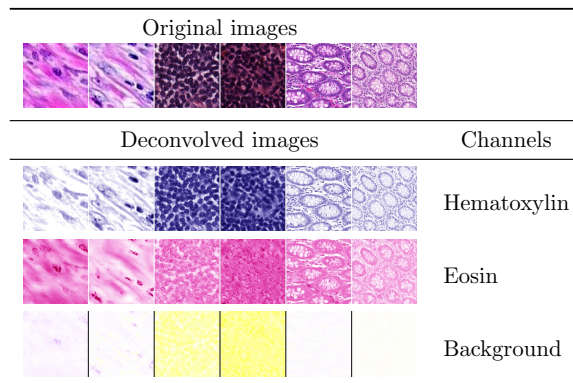


Fig. 3: Colour deconvolution via Ruifrok and Johnston’s method: samples of original (RGB) and deconvolved images. Deconvolved channels are in pseudo-colours..

orientation  $0^\circ$ ,  $45^\circ$ ,  $90^\circ$  and  $135^\circ$  ( $5 \times 3 \times 4 = 60$  features). Discrete Fourier transform (DFT) normalisation was applied for rotation invariance [22].

*Gabor filters* (Gabor). Mean and standard deviation of the magnitude of transformed images from a bank with four frequencies and six orientations ( $2 \times 4 \times 6 = 48$  features). Rotation invariance was obtained via DFT normalisation.

*Local Binary Patterns* (LBP). Concatenation of rotation-invariant (‘ri’) LBP histograms computed at resolution 1px, 2px and 3px using non-interpolated eight-pixel neighbourhoods as detailed in [23].

*Hybrid methods*. Marginal colour versions of Gabor, GLCM and LBP were also obtained by applying the corresponding grey-scale descriptor to each colour channel separately and concatenating the resulting features. These are indicated as ‘MargGabor’, ‘MargGLCM’ and ‘MargLBP’ in the remainder.

*Pre-trained convolutional networks*.  $L_2$ -normalised output of the last fully-connected layer from the following pre-trained networks: ResNet-50 and ResNet-101 [24], VGG very deep 16 and VGG very deep 19 [25].

### 3 Results and discussion

Each dataset was analysed for a combination of pre-processing and image descriptor, the accuracy being estimated via split-sample validation with stratified sampling at the image level<sup>4</sup>. The random subdivision into train and test set was repeated a hundred times and the results averaged. Classification was based on the 1-NN rule with  $L_1$  distance. We used a train ratio (i.e. fraction of images used for training)  $f = 1/4$  and  $f = 1/8$ , and the results did not show significant deviation in the overall trend. The best and second-best combinations are shown in Fig. 4. Pre-trained ResNet50/ResNet101 performed best or second-best in eight cases out of 14 (accuracy range of best configurations 71.56% –

<sup>4</sup> Complete results available at [https://drive.google.com/drive/folders/1bc1m0\\_RCQppfbrCjqjWlF50YoZNLWbW4?usp=sharing](https://drive.google.com/drive/folders/1bc1m0_RCQppfbrCjqjWlF50YoZNLWbW4?usp=sharing)

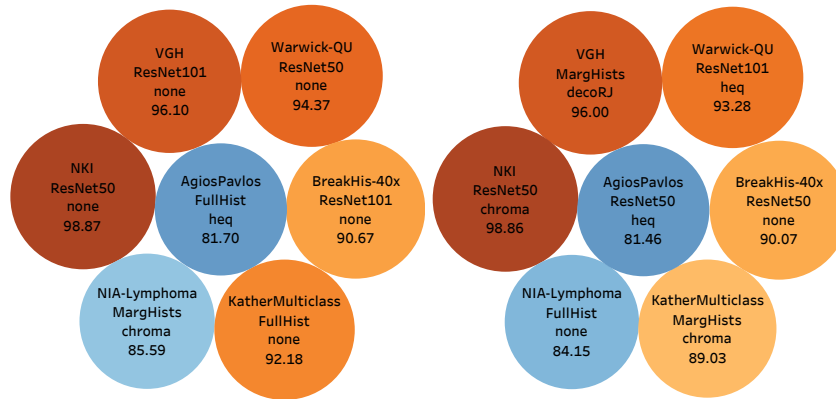


Fig. 4: Best (left) and second-best combinations (right) for each dataset. Each circle corresponds to a dataset, with its descriptor, pre-processing and accuracy. Colours reflect accuracy (brown = best, blue = worst). Results obtained for  $f = 1/4$ .

98.81%), followed by joint and marginal colour histograms (six cases, accuracy range 80.09% – 98.16%). As for colour pre-processing, doing nothing was the best or second-best option in seven cases out of 14, followed by heq and chroma.

Table 1 shows the difference to the baseline (colour pre-processing vs. no colour pre-processing) by descriptor and pre-processing method. Colour pre-processing resulted in a loss of accuracy in most cases – in particular, methods that rely on colour responded negatively to colour pre-processing. Colour transfer via Macenko’s and Reinhard’s methods did not show a clear trend as for the effects of the target image used. Note that the non-histological target image (T1) gave better results than the histological ones (T2–T4) in some cases.

By contrast, the texture-based methods proved more resilient to colour pre-processing. In this case there was even a noticeable gain in accuracy in some combinations descriptor/pre-processing method: marginal colour texture descriptors (i.e. MargGabor, MargGLCM and MargLBP) responded positively both to ‘chroma’ normalisation and colour deconvolution. This suggests that texture features provide complementary information when extracted separately from each of the hematoxylin, eosin and background channels.

## 4 Conclusions

Colour pre-processing produced a noticeable loss of accuracy in the classification of histological samples in most cases, particularly when used along with image descriptors that rely on colour. This is consistent with [10], but differs from [9]. Some pre-processing methods (i.e. chroma and decoRJ) had positive effects when coupled with certain texture descriptors, specifically MargGabor, MargGLCM and MargLBP. This is a novel finding that should be better explored and validated in future studies.

Table 1: Effect of colour pre-processing: difference to the baseline broken down by image descriptor and colour pre-processing method. Colour reflects accuracy (brown = best, blue = worst). Values are averaged over the seven datasets;  $f = 1/4$ .

Descriptor	Colour pre-processing method														AVG
	none	chroma	gmv	heq	Macenko (T1)	Macenko (T2)	Macenko (T3)	Macenko (T4)	Reinhard (T1)	Reinhard (T2)	Reinhard (T3)	Reinhard (T4)	decoRJ		
FullHist	0.00	-8.10	-3.06	-1.77	-5.84	-5.26	-6.28	-5.02	-42.65	-42.65	-42.65	-42.65	-31.50	-18.26	
MargHists	0.00	0.59	-5.82	-24.50	-7.08	-4.70	-4.18	-5.05	-27.32	-28.25	-27.00	-30.20	-6.90	-13.11	
Gabor	0.00	-0.42	1.16	-2.78	-2.11	-1.84	-1.68	-2.03	-2.18	-1.45	-1.66	-1.22	-0.28	-1.27	
GLCM	0.00	0.96	0.52	-3.41	-0.30	-1.64	-0.16	-2.67	-1.50	-2.68	-1.02	-6.82	-2.53	-1.63	
LBP	0.00	3.64	-0.36	-0.23	0.39	-0.16	0.01	-0.09	1.48	-1.12	-0.36	-0.56	-0.47	0.16	
MargGabor	0.00	2.31	1.65	-0.52	2.04	-1.44	-1.73	-1.96	-1.75	-0.93	-2.42	-1.88	5.80	-0.06	
MargGLCM	0.00	4.71	0.78	-1.91	2.75	-3.95	-3.11	-5.47	-0.68	-3.66	-1.32	-6.97	4.66	-1.09	
MargLBP	0.00	4.40	0.40	-0.17	2.02	-0.67	-1.12	-0.81	3.70	-0.26	-0.42	-1.56	2.93	0.65	
ResNet50	0.00	-2.24	-1.39	-1.32	-1.91	-2.48	-1.96	-2.31	-22.72	-25.56	-24.50	-26.41	-14.71	-9.81	
ResNet101	0.00	-3.83	-0.39	-1.63	-2.93	-1.50	-1.35	-2.88	-24.64	-26.01	-25.58	-26.74	-17.31	-10.37	
Vgg16	0.00	-2.93	-1.13	-1.04	-3.90	-0.87	-1.21	-1.30	-20.17	-22.79	-21.90	-23.38	-14.20	-8.83	
Vgg19	0.00	-1.05	-1.14	-2.26	-1.29	-3.58	-1.75	-4.81	-19.50	-20.47	-20.06	-20.42	-12.29	-8.36	
AVG	0.00	-0.16	-0.73	-3.46	-1.51	-2.34	-2.04	-2.87	-13.16	-14.65	-14.07	-15.73	-7.23	-6.00	

In conclusion, our results suggest that the use of colour pre-processing for patch-based classification of H&E images should be considered with care. In particular: 1) no pre-processing may provide better results than pre-processing in most cases; 2) the selection of the pre-processing procedure should always be evaluated in conjunction with the image descriptor(s) used.

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